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(54) Title: IDENTIFICATION METHOD FOR VERIFYING THE AUTHENTICITY OF AN OBJECT

(54) Bezeichnung: NACHWEISVERFAHREN ZUR PRÜFUNG DER ORIGINALITÄT EINES OBJEKTS

(57) Abstract: The invention relates to an identification method for verifying the authenticity of an object during which the action of human saliva or constituents contained therein upon a marker, which is bound to the object or contained therein, generates a specific signal, whereupon this signal is evaluated.

(57) Zusammenfassung: Die Erfindung betrifft ein Nachweisverfahren zur Prüfung der Originalität eines Objekts, bei dem durch die Einwirkung von humanem Speichel oder darin enthaltenen Bestandteilen auf einen Marker, der mit dem Objekt verbunden bzw. in ihm enthalten ist, ein spezifisches Signal erzeugt und dieses Signal ausgewertet wird.

### Identification method for verifying the authenticity of an object

Because recent years have shown an increase in the counterfeiting of brand products and the manipulation of information directly connected with the product, e.g., expiry dates on medicines, there is a pressing need for selective, simple methods for verifying the authenticity of an object or information connected with the object, where such methods must also be able to be carried out quickly and they must be hard to counterfeit and hard to circumvent or manipulate.

In the state of the art, various selective methods for verifying the genuineness of objects are already known.

For example, a selective method for verifying the genuineness of objects is described in WO 98/33162 which is based on the selective interaction between a print molecule and a molecularly imprinted molecule. In this method, the component to be verified (print molecule) is imprinted in a polymer, which subsequently contains three-dimensional impressions (molecularly imprinted molecule) of the molecule. Proof of the authenticity is based on the (more or less) specific recognition of the imprinted molecule by a polymer.

From each of EP-A-0327163, US-A-5,429,952 and WO 95/06249, the use of specific antibodies in the framework of an immunoassay for verification of genuineness is known. WO 87/06363 uses macromolecular test components, such as proteins and nucleic acids, for example, for marking products, wherein the specific identification is given with marked antibodies in the case of the proteins and with correspondingly marked nucleic acid probes in the case of the nucleic acid.

Detrimental in these methods is that the formation of a complex by using two complementary binding partners does not lead directly to a signal that can be evaluated, and therefore verifying the authenticity requires a sequence of consecutive steps, e.g., for separating a reagent that is not specifically bound, in order to obtain a specific signal.

The various steps prolong the analyses and increase the costs. Moreover, in the case of a verification of authenticity that should be performed quickly, independently of instruments, and even by untrained persons, a swift and simple method that is not subject to mistakes is particularly desirable.

PCT/EP01/00764 describes an identification system for verifying authenticity, in which the object is marked, for example, with an enzyme substrate, and this is brought about to react with the enzyme in an appropriate test device, wherein the products of the reaction result in an identifiable signal, directly or after being combined with an indicator system. This method allows the verification to be carried out swiftly with a test device that is relatively simple to construct.

In contrast, the object of the present invention consists of further simplifying the test method and replacing the test device with a simple, economical and efficient testing agent that is available to the user at all times.

Saliva meets these stipulations in an ideal manner, being a testing agent that is easily accessible and available at all times. In the present invention, specific, natural ingredients of human saliva function as a necessary component or a mixture of components for the successful performance of a verification of authenticity with an appropriately marked object, wherein generation of a specific signal confirms the authenticity of the marked, tested object.

The above-mentioned object is consequently solved according to the invention by an identification method for verifying the authenticity of an object, which is characterised in that the action of human saliva or components contained therein on a marker that is bound to the object or contained therein generates a specific signal, and this signal is evaluated.

One preferred fundamental embodiment of the identification method according to the invention is based on the reaction of a substrate (and optionally co-substrate) with a suitable enzyme, wherein the products of the reaction, directly or after combination with an indicator system, result in a specific, identifiable signal, which confirms the authenticity (genuineness) of the object.

In this case, the catalyzing enzyme is preferably contained in the saliva. The other components necessary for generating a signal, such as a substrate or, optionally, a co-substrate, indicator system components and other enzymes necessary for generating the signal, for example, that are not contained in the saliva are present in the marker which is bound to the object or contained therein.

The enzyme  $\alpha$ -amylase in saliva is preferably used as the saliva enzyme. This enzyme preferably cleaves starch or starch derivatives, such as hydroxyethyl starch, for example. The starch molecule

is split by the  $\alpha$ -amylase, with statistical distribution of the cleavage sites, into oligomerically branched saccharide fragments and, to some extent, also already into the monosaccharide glucose. These glucose units then serve as the substrate for the subsequent enzymatic reaction that leads to generation of the signal. Optionally, for example, the enzyme 1,6-glucosidase can also be provided on the object, in order to catalyze an additional decomposition of oligomers into glucose units.

Preferably the enzymes glucose oxidase, peroxidase, a substrate, such as tetramethylbenzidine (TMB), for example, a peroxide, for example, hydrogen peroxide, are immobilized on the marked object. In the enzymatic decomposition of the starch into glucose units, which is catalyzed with the help of the  $\alpha$ -amylase from saliva and optionally provided 1,6-glucosidase, a signal, for example, a blue one, is generated by the enzymatic reaction of the enzymes glucose oxidase and peroxidase with the help of, for example, TMB and  $H_2O_2$ .

The components that are not contained in saliva but that are necessary for the reaction are applied to the object in suitable matrices. Hydrophilic and hydrophobic paints, colouring materials or polymers are such matrices. Depending on the matrix, the individual components can be mixed directly into these matrices. Alternatively, the components that are not contained in saliva but that are necessary for the reaction can be introduced in a protected form, e.g. enclosed in microcapsules or microparticles. In connection with the present invention, microcapsules are understood to be microcasings of various materials, e.g. gelatine, starch, cyclodextrin or chitosan, which are filled with the particular ingredient or ingredients desired. On the other hand, the term "microparticles" describes porous three-dimensional structures that can likewise consist of gelatine, starch, cyclodextrin or chitosan, but also of polymers or copolymers. The microparticles contain one or more ingredients either embedded in the three-dimensional porous structure or bound to its surface by adsorption.

In a second preferred fundamental embodiment, the marker therefore includes microcapsules or microparticles, which are broken up by the action of human saliva or constituents contained therein, i.e. opened, cleaved or influenced in another way, e.g. by physical interaction, to the effect that enclosed or chemically bound or adsorbed ingredients or constituents of the microcapsules or microparticles are released.

Microencapsulation is a process in which the desired materials are enclosed in capsules, preferably of a size of roughly 1 - 100  $\mu m$ . In this process, these materials are completely and thoroughly

enveloped. This enveloping first serves to protect the enclosed components from outside influences (e.g., atmospheric oxygen, moisture, other chemical reaction partners) and, furthermore, it serves the selective release under whichever conditions are suitable. In a loose form or simply present free in the matrix, many materials would quickly lose activity, and in this way, they can be kept enclosed so that they remain capable of reacting until the desired point in time.

Microcapsules are produced by means of various technologies that are offered commercially by various specialists and that are widely used in the foodstuffs and cosmetics industries, in particular.

Processes such as extrusion / spraying / cooling, extrusion / cutting, coating / extrusion / grinding, coacervation or emulsion / extrusion and other processes are used to produce microcapsules. The microcapsules or microparticles can be located in a medium or a matrix, which likewise can optionally contain components for signal generation.

The medium or the matrix in which the microcapsules or microparticles are introduced, for example, by being mixed in, consists, for example, of materials that are normally used in printing technology. These are commercially available paints such as UV paints, inks, water soluble paints, e.g. Hydrokett HK P061 from Akzo Nobel, solvent-based varnishes or commercially available adhesives, e.g. hot-melt glue. These materials are applied to the corresponding surfaces using customary print techniques, such as screen printing, rotogravure printing, flexographic printing or offset printing. The materials are dried using UV drying, for example, which results in polymerization of the materials.

In both embodiments, the reaction that generates the signal is started by the user who is carrying out the verification by simply moistening the marked object, for example, a product, a product package, a customs stamp, a label or an object with value imprint at the place of the marking with saliva or a liquid that contains at least one saliva constituent, preferably one or more saliva enzyme(s). Preferably, the signal is created directly on the marked object. Alternatively, however, transfer of signal-generating components to a separate test device, as disclosed in DE 100 02 819 A1, is also possible.

The invention consequently provides an identification system for verifying the authenticity of an object, wherein at least one constituent that is necessary for the identification is contained in saliva and at least one component is available in a marker that is bound to the object or contained therein.

Some non-restricting examples of suitable enzymatic activities in human saliva are amylases, preferably  $\alpha$ -amylases, lysozyme, peroxidase and lactoferrin. These can be used as saliva or in the form of a liquid that contains one or more of these enzymes.

The capsules or particles that are to be used in the second fundamental embodiment of the method according to the invention have a size and / or wall thickness that is adjusted to the specific requirements for optimal signal generation and convenient marking of the object (e.g. using printing processes). The size of the capsules or particles is preferably 1 - 100  $\mu\text{m}$ . For manufacturing and filling the capsules, common methods can be used (see, for example, Carbohydrate Polymers 1994, 24 (4) 295-300, Advances in Polymer Science 136, Springer Verlag, 1998; Abe, Albertson et al.). Suitable capsules that comply with a desired requirements profile can also be obtained from firms specializing in this area.

The technology for encapsulation or for enclosing ingredients also depends for the most part on the nature of the ingredients. To design the encapsulation technology with the intended ingredients for signal generation in a manner that is as uncomplicated as possible, it can be built on common technologies for the encapsulation of other ingredients. For example, the ingredients that are of interest here can also be encapsulated, or enclosed or bound in microparticles in combination with auxiliary agents.

The preferred base materials for such microcapsules or microparticles are substances which can serve as substrates for saliva enzymes, such as those mentioned above, for example. The enzyme-substrate reaction then leads to an opening of the microcapsules and / or to decomposition of structures of the microparticles and to the release of the enclosed or otherwise bound or immobilized components for signal generation. These base materials can be modified and / or supplemented or combined with auxiliary agents as needed in such a way that desired chemical or physical properties concerning the application intended here are optimised. Among these properties are, for example, a sufficient enzymatic divisibility due to the saliva enzymes, sufficient long-term chemical stability on the marked object, sufficient suitability with respect to the technical processability and suitable solubility characteristics in technical processing and in the framework of the testing process with saliva. In order to minimize or rule out solubility of the capsules or particles by water alone, meaning without the action of enzymes specific to saliva, cross-linking or other modification of the capsule or particle surface can be carried out, for example. Such cross-linking can be brought about with polypeptides, for example. Corresponding modifications of the capsule or particle material also lead

to decomposition of the capsules or particles that takes place swiftly and under realistic conditions, meaning preferably at room temperature, and consequently guaranteeing that signal generation is satisfactorily swift and intensive.

Preferred base materials are the polymer chitosan and derivates thereof that are divisible by the saliva enzyme lysozyme or poly-L-lysine.

Likewise preferred are starch-based materials that are divisible by amylases, preferably  $\alpha$ -amylases. Such starch-based materials comprise starch and various types of modified starch derivatives, for example, a hydroxyalkyl starch, e.g., hydroxyethyl starch, or cyclodextrin, whose chemical and physical properties can be adapted to the intended use.

The use of different types of capsules or particle types, for example, on the basis of different chemical modifications and / or wall thicknesses, makes possible decomposition that is staggered in time. In this way, it is possible to release different ingredients from different capsule and / or particle types successively, and thereby to generate signals following each other in time and to form signal cascades. In this way, imitation of the signal generation is made considerably more difficult.

The signal generated according to the invention can be detectable in different ways. Preferably, it will be a signal that can be perceived directly by one of the human senses. Particularly preferred is a visual, particularly colour, signal. The visual signal can also be a fluorescent, luminescent or phosphorescent signal that is only visible under suitable conditions, e.g. illumination with UV light or in the dark. Alternatively, the signal could also be perceptible with the sense of smell or taste.

In an alternative embodiment, a physical, including visual, signal is generated, that can be evaluated by a suitable measurement instrument. Such instruments could be fluorometers, reflectometers, conductivity meters, etc. for example.

In a further embodiment, a signal is generated that can be evaluated by another non-instrument test device, for example, following the test device in DE 100 02 819 A1.

The signal can be generated by the release of a single component, by the release of various components or by the interaction or reaction of different components.

In one embodiment, all components necessary for the signal generation, with the exception of the saliva components, can be enclosed in the microcapsules or microparticles and selectively released for signal generation. In another embodiment, which in any case requires more than just one component for signal generation, only part of the components is enclosed in microcapsules or microparticles, while the other part of the components can be present free in the surrounding medium, for example, a printing ink or plastic matrix (see above).

A further aspect of the present invention also relates to methods and compositions for signal generation, in which a specific signal is generated by the action of mechanical shearing forces and / or solvents, particularly organic solvents, on the marked object.

In one embodiment of this aspect of the invention, microcapsules or microparticles are used that preferably can be opened by the action of mechanical shearing forces. Suitable base materials for capsules of this kind are, for example, waxes, alginates, casein, gelatine, or liposomes. These base materials can be modified and / or supplemented or combined with auxiliary agents as needed in such a way that desired chemical or physical properties concerning the application intended here are optimised. Among these properties are, for example, sufficient long-term chemical and mechanical stability on the marked object and technical processability. The capsules have a size and wall thickness that are adapted to the particular requirements for optimal signal generation and convenient marking of the object and that are adapted to the special use. The capsule size generally lies in a range of roughly 1 - 100 µm. Suitable capsules with the especially desired properties can be obtained from specialized firms. Components that generate the signal are enclosed in the capsule, and these components are released after the capsules are opened by shearing forces, generating a specific signal according to the mechanisms described in the following. This type of signal generation by mechanical action on the marked object can be used for various purposes.

In one embodiment, the authenticity of an object marked in this way is examined in a simple and uncomplicated manner. The mechanical shearing forces that, for example, are generated by rubbing an article, for example, the tester's fingernail, on the marked object, for example, a label, serve here as a simple and free test agent that is available at any time. Such an identification method can be used in addition to or as an alternative to the identification method described above that uses human saliva. The general explanations relating to this that were made on the manufacture and use of microcapsules or microparticles, as long as they do not relate especially to the opening of the capsules by saliva enzymes, also generally apply for the method addressed here with capsules that

are to be opened mechanically. In particular, this also applies to the different mechanisms and systems of signal generation described in the following.

In a second embodiment, the capsules that are described here and that are to be opened mechanically are used to identify the manipulation of a marked original product.

For example, counterfeiters attempt to remove labels from used original products and attach them to imitations. In order to prevent this, capsules of this kind, for example, could be introduced into the adhesive matrix on the back of the label. In the attempt to remove the label by tearing it off of the original product, the capsules open as a result of the shearing forces that arise and a signal, e.g. a colouration, is generated.

In an alternative procedure, counterfeiters attempt to change the labels with the help of benzine or other, preferably organic, solvents.

In order to identify such a manipulation, the label should be changed by the manipulation in such a way that it can be recognized as manipulated. One possibility for this consists in the introduction of signal-generating components, for example, pigment particles, in, for example, the adhesive matrix of the label, which result in a signal when influenced by solvents as a result of physical or chemical interaction. For example, in one embodiment, lipophilic dyes, preferably as dispersions, can be introduced into the matrix. Manipulation for the purpose of detaching the label results in the dyes dissolving in the matrix, consequently causing a signal which identifies the label as modified.

For example, the lipophilic dye  $\beta$ -carotene can be introduced into the adhesive matrix as a dispersion in the form of the smallest drops. Under the influence of a lipophilic solvent, such as benzine, for example, the drops in the dispersion, which are barely visible, are distributed uniformly across the entire surface and consequently generate a colouration that marks the label as manipulated. These marking and signal generation methods for protection from counterfeits and manipulations are preferably used alongside the identification methods described above for verifying the authenticity of an object, preferably with the use of human saliva as the test agent.

In the following, different systems and components for signal generation are described by way of example. In the second fundamental embodiment of the identification method for verifying the authenticity of an object using human saliva according to the invention, at least one of the

components necessary for signal generation by means of the action of human saliva or one of the components contained therein, e.g. the saliva enzymes mentioned above, is to be released from microcapsules or microparticles. The systems and components that can be used, for example, catalysts, in particular enzymes, reaction partners, indicators or also the environment that is modified by the release of components in the medium, can vary greatly and can therefore also generate a very wide range of signals. Accordingly, the special embodiments explained in the following are in no way to be understood as a limitation of the invention. For a person skilled in the art, suitable modifications of or alternatives to the embodiments that are described would be easily apparent.

#### Direct signal generation using dyes

Colouring components, such as colouring pigments, for example, carotenoids, azorubine or yellow-orange, or other pigments, such as cobalt blue or cobalt green, are enclosed in microcapsules or microparticles. In the capsules or particles, the dyes are present in compact form, while they are relatively uniformly distributed in the medium after the release. This modified distribution pattern supplies a visual signal that is preferably perceptible as a colouration on the object.

In a further embodiment, the colouration of pigments is entirely or partially shielded or modified by the microcapsules or particles. The release of the pigments reduces this shielding and consequently results in a visual signal.

#### Direct signal generation using indicators

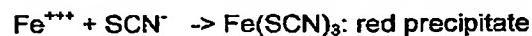
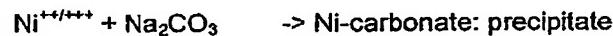
Substances that function in known reactions as pH indicators are enclosed in microcapsules. When the indicator is released, a change in the pH arises that is either caused by the saliva or stipulated by introduced substances, for example, salts in the surrounding medium, for example, a liquid medium such as printing ink or a matrix material, and so results in a sudden change in the colour of the indicator. In this process, the indicator is encapsulated, for example, in a neutral environment, and there is an acidic or alkaline environment in the medium, which results in a sudden change in the colour. For example, if phenolphthalein or litmus with a defined pH is encapsulated in a neutral environment, and if there is an alkaline pH in the medium after the release, the result is a sudden change in colour to red.

Signal generation as a result of inorganic reactions

For example, salts or salt solutions, thus ionic solutions, are separately encapsulated in different capsule collectives. As used here, the term capsule collective comprises those homogenous capsules that encapsulate identical material. After being released, ions come into contact that form the precipitating, preferably coloured, precipitates, coloured colloids or suspensions or coloured solutions, for example, complex compounds. These coloured products can be seen as a visual signal on the marked object. Alternatively, it is also possible for one of the components to be present in the surrounding medium without being encapsulated.

In one typical example, a solution of red potassium prussiate ( $K_3[Fe(CN)_6]$ ) and a solution of ferrous (II) salt or a solution of yellow potassium prussiate  $K_4[Fe(CN)_6]$  and a solution of ferrous (III) salt are separately encapsulated. After the capsules open, dissolved Berlin blue is formed colloidally.

Following are a few additional, non-restricting examples for ionic reactions with coloured reaction products, preferably coloured precipitates:



In each of the reactions given as examples, components are encapsulated or enclosed in microparticles, whereby these components lead to corresponding precipitates or reaction products when they come into contact with other components. In this process, at least one ionic component is encapsulated or enclosed in microparticles, while the other ionic component either is likewise enclosed or can be present free in the surrounding medium.

Signal generation as a result of organic reactions

Fundamentally, all reactions in which at least two reaction partners contribute to the formation of coloured reaction products and thereby to signal generation are suitable. As used here, the term

"organic reactions" should also include reactions in which only one reaction partner is an organic molecule. On the other hand, all reaction partners or at least one of them can be enclosed in microcapsules or microparticles, so that all components that are necessary for a reaction that generates a signal come into contact only after the release from the microcapsules or microparticles. Preferably, the reaction should run spontaneously as a result of the contact of the reaction partners in the medium, meaning in particular even without considerable energy being supplied from the outside.

A few special examples of suitable organic reactions are the formation of triphenylmethane colouring substances or the formation of methylene blue, which arises from a leuco compound as the result of a redox reaction, or the formation of azo pigments. Preferred examples are the formation of indigo blue from indigo with dithionite or the diazotization of aniline to aniline yellow.

An example for the formation of a coloured complex between a metal ion and organic ligands is the Ni-diacetyl dioxime complex, which results in an intense red precipitate. A further example is the reaction of many phenols with Fe(III)-chloride under the formation of coloured complexes.

A preferred group of organic reactions is that between electron acceptor compounds and electron donor compounds, which lead to the formation of coloured charge-transfer complexes. Preferred as electron donors are aromatic and hetero-aromatic compounds with electron-supplying substituents, such as aromatic amines (aniline), alkylbenzenes (Friedel-Crafts acylation). To be considered as electron acceptors are, for example, substituted aromatics and hetero-aromatics with electron-drawing substituents, such as aromatic ketones, as well as other organic or inorganic electrophilic compounds, such as known Lewis acids (trimethylborane, magnesium ions, trimethyl cations). The binding partners for charge-transfer complexes can also be inorganic or organic ions or radicals.

Examples of charge-transfer complexes of this kind are the coloured addition compounds of p-benzoquinone and hydroquinone with, for example, dimethylbenzene or trimethylbenzene, or the complex that is the result of the reaction of 3-(dimethylamino)benzoic acid with 4-(dimethylamino)antipyrine in the presence of hydrogen peroxide and peroxidase (US 4,321,397). A further example is the colour reaction of the almost colourless aquo complex of  $\text{Fe}^{3+}$  with the addition of the appropriate ions in suitable concentrations to the yellow chloro-complex or to the red

rhodanide complexes. When used in the identification method according to the invention, at least one reaction partner for the addition compound is present in encapsulated form or in microparticles.

#### Signal generation as a result of catalyzed reactions

In this embodiment, the catalytic component is first separated from the reaction partners of the chromogenic reaction. In this process, it is possible to encapsulate or enclose in microparticles either only the catalytic component or the catalytic components and one or all reaction partners. Alternatively, the catalytic component is present in the matrix or the medium, whilst one or more reaction partner(s) is / are encapsulated. The encapsulation or enclosure of catalytic components makes it possible for the reaction partner(s) to be already present in the medium or matrix, and for the release of the catalytic component to start the reaction. Because catalysts are often effective in very small amounts, this provides the advantage that the release of even just small amounts of the catalytic substance can trigger the chromogenic reaction. This is very advantageous in the sense of a swift reaction and therefore swift generation of the signal. An example for a catalytic reaction is the acid-catalyzed dimerization of certain starting compounds by Friedel-Crafts acylation. This leads to the formation of intermediary stages that are catalytically oxidized by atmospheric oxygen into the final stage of the colouring substance.

#### Signal generation as a result of enzyme-substrate systems

The signal generation preferably takes place with chromogenic substrates, whereby the enzyme, for example, is located in a capsule collective, the substrate and, optionally, additional chromogenic components or indicator components are located in at least one additional capsule collective or in the surrounding medium. In the case of enzymes that are formed from an apoenzyme and a coenzyme, the coenzyme can be located together with the apoenzyme in one capsule collective or separate from it. In the latter case, it can be alone in a capsule collective or in the medium, or together with the substrate in a capsule collective or the medium. The cleavage of the capsules leads to contact among the components that were previously separate, and consequently facilitates the reactions that result in the signal generation.

Substrates used according to the invention are all chemical compounds whose enzymatic reaction leads directly to a detectable signal-generating product, for example, a coloured product, or whose

reaction leads to a primary product that, when combined with an indicator system, leads to a signal-generating secondary product.

Such substrates are known for many common enzymes or can be manufactured according to known processes. In this way, for example, suitable substrates of carbohydrate-specific enzymes, proteases, nucleases, lipases, oxidases, peroxidases, oxidoreductases, transferases, hydrolases, lyases and kinases can be used in accordance with the invention. More specific non-restricting examples are substrates of amylases, e.g.  $\alpha$ -amylases, oxidases, e.g. cholesterol oxidase, uricase, peroxidases, phosphatases, e.g. alkaline phosphatase, galactosidases, glucosidases, DNAses, RNases, lysozyme, lactoferrin, etc. Examples of chromogenic substrates are p-nitrophenyl phosphate, which is reacted by alkaline phosphatase into a yellow reaction product, or tetramethylbenzidine (TMB), which is reacted into a blue reaction product in the presence of hydrogen peroxide. One fundamental procedure for the production of chromogenic substrates consists, for example, of coupling a latently chromogenic group to a larger molecule, which is split off by the enzymatic reaction and then results in a visual signal.

In one preferred embodiment, suitable substrates for saliva enzymes, such as amylases, peroxidase, lysozyme or lactoferrin, for example, are selected. This offers the advantage that the enzymatic activity of human saliva or of the components contained therein can both open the microcapsules / particles and start the signal-generating reaction.

If no primary reaction product of the enzymatic reaction results in a directly detectable signal, the or a reaction product can be reacted with components of a suitable indicator system, with formation of a detectable, for example, coloured or fluorescent, secondary product. In the state of the art, a number of suitable indicator systems are known for different known enzyme substrate systems. If, for example, an oxidation agent, such as hydrogen peroxide, for example, results from the enzymatic reaction, this can oxidize a suitable reaction partner, for example, yellow potassium prussiate, which results in a strongly coloured product. In a similar way, a reduction agent that has already resulted can also be further reacted. Alternatively, the or a primary reaction product can serve as a substrate for a second enzyme, which then results in a coloured or otherwise detectable reaction product. A further possibility consists of the or a reaction product reacting with a specific binding partner, which can then generate a signal after the coupling.

One special example of an enzyme-substrate system according to the invention is the encapsulation of the enzyme peroxidase in a capsule collective and of suitable substrate components, preferably a prepared TMB substrate (e.g. TMB ONE from Biotrend, Cologne, Germany) in another collective. The prepared TMB substrate is reacted into a blue end product according to a known reaction mechanism.

Signal generation by means of a combination of specific binding partners

Inclusion of at least one of two or more specific binding partners in microcapsules or microparticles results in binding taking place after the release, and then directly or indirectly, with the help of appropriate markers, generation of a detectable signal.

One example of this is the separate encapsulation of single-strand nucleic acid molecules, for example, DNA or RNA or hybrids of these, which complement one another, which hybridize together into a double-strand after the release, whereby a specific signal is generated as the result of the hybridization. This signal can, for example, be generated by intercalation of a colouring substance or fluorescent colouring-substance, e.g. ethidium bromide, into the double-strand. Alternatively, each of the single strands can be provided with marker molecules, which do not come into contact with each other until after the hybridization and which can then generate the signal. Such markers are known in the state of the art (e.g. can be obtained from November AG, Erlangen). To protect the nucleic acid from UV radiation, auxiliary agents, e.g. zinc oxide, can be furthermore added.

A further example is the separate inclusion, preferably encapsulation, of proteins which are complementary to each other or of other molecules, e.g. antigen and antibody or receptor and ligand, or the inclusion of at least one of the respective complementary binding partners. Again in this case, at least one binding partner must also be provided with a marker, which does not result in a signal until after the binding partners have been united.

Signal generation by means of a combination of more than one mechanism

The cited methods for signal generation can also be combined with one another in any fashion, whereby components can be enclosed in different capsule collectives or particle collectives and

released, separately or together. In this way, multiple-stage reaction cascades are also possible, so that several different signals can be generated at the same place on the marked object, simultaneously or staggered in time, and the complex signal pattern that emerges can be evaluated. Additionally or alternatively, it is also possible to generate several different signals at different locations on the object. Signal generation that is more complex increases the safety in the face of possible counterfeits.

The following examples are meant to explain the identification method according to the invention in more detail.

#### EXAMPLE

Starch capsules filled with silver nitrate solution (10 mg/ml) are mixed into the paint Hydrokett HKPO61 from Akzo Nobel with a volume content of 5 percent by weight. In addition, a sodium chloride solution (7%), not encapsulated, in a ratio 1/10 up to 1/4 is mixed into the paint. The paint is subsequently applied to the surface of the paper using customary technological methods, in a coat with a thickness of several µm and then air-dried.

When moistened with saliva, this diffuses into the paint matrix and the capsules open. The released silver nitrate solution, together with the chloride ions, forms a black colouration, which is triggered by a precipitate.

**PATENT CLAIMS**

1. Identification method for verifying the authenticity of an object, characterised in that the action of human saliva or constituents contained therein upon a marker, which is bound to the object or contained therein, generates a specific signal, and this signal is evaluated.
2. Identification method according to Claim 1, characterised in that an enzymatic activity contained in human saliva acts on the marker and at least one of the reactions catalyzed by this enzymatic activity directly or indirectly leads to the generation of a specific signal.
3. Identification method according to Claim 2, characterised in that the enzymatic activity is the activity of lysozyme, lactoferrin, of an amylase or a peroxidase or a combination thereof.
4. Identification method according to Claim 2 or 3, characterised in that the marker contains at least one substrate for the enzymatic activity contained in human saliva and at least one reaction catalyzed by this activity results in at least one reaction product, which, as such or in combination with a suitable indicator system, generates a specific signal.
5. Identification method according to one of the Claims 1 - 4, characterised in that the marker comprises microcapsules and / or microparticles, which are opened by the action of human saliva or constituents contained therein, so that at least one enclosed ingredient and / or one bound component of the microcapsules and / or microparticles is released and directly or indirectly generates a specific signal.
6. Identification method according to Claim 5, characterised in that the microcapsules and / or microparticles comprise materials on the basis of starch or chitosan, which are opened by the action of human saliva or components contained therein.
7. Identification method according to Claim 6, characterised in that the starch-based materials comprise or consist of starch, modified starch or starch derivatives.

8. Identification method according to Claim 6 or 7, characterised in that the starch-based materials comprise or consist of a hydroxyalkyl starch, e.g. hydroxyethyl starch, or cyclodextrin.
9. Identification method according to one of the Claims 5 - 8, characterised in that at least one released ingredient or a component of the microcapsules and / or microparticles enters into a chemical or physical-chemical reaction and leads to the formation of at least one reaction product that directly or indirectly generates a specific signal.
10. Identification method according to Claim 9, characterised in that the chemical reaction is an enzyme-catalyzed reaction.
11. Identification method according to one of the Claims 5 - 10, characterised in that at least one released ingredient or component of the microcapsules and / or microparticles is a catalyst and, after the release, catalyzes a reaction that leads to the formation of at least one reaction product that directly or indirectly generates a specific signal.
12. Identification method according to Claim 11, characterised in that the catalyst is an enzyme.
13. Identification method according to one of the Claims 9 - 12, characterised in that the signal is generated by the combination of at least one reaction product with a suitable indicator system.
14. Identification method according to one of the Claims 5 - 8, characterised in that the release of the at least one ingredient or component of the microcapsules and / or microparticles directly generates a specific signal.
15. Identification method according to one of the Claims 5 - 14, characterised in that a microcapsule or a microparticle encloses several ingredients or components.

16. Identification method according to one of the Claims 5 - 15 characterised in that the marker comprises two or more microcapsule collectives and / or microparticle collectives that contain different ingredients or constituents and / or that are opened under different conditions.
17. Identification method according to Claim 16, characterised in that some or all of the components necessary for generating the signal, with the exception of the saliva components, are distributed among two or more of the marker's microcapsule collectives and / or microparticle collectives.
18. Identification method according to one of the Claims 5 - 9, characterised in that at least one of two specific binding partners or both is / are enclosed in microcapsules or microparticles, and that these bind together after the release of the at least one binding partner, wherein a specific signal is generated as a result of the binding.
19. Identification method according to Claim 18, characterised in that the binding partners are complementary single-stranded nucleic acid molecules that hybridize with each other into a double-strand after the release, wherein a specific signal is generated as a result of the hybridization.
20. Identification method according to Claim 19, whereby the signal is generated by the intercalation of a dye or fluorescent dye into the nucleic acid double-strand.
21. Identification method according to Claim 19 or 20, characterised in that auxiliary agents, e.g. zinc oxide, are used to protect the nucleic acid from UV radiation.
22. Identification method according to one of the Claims 5 - 21, characterised in that the microcapsules and / or microparticles are located in a medium or matrix.

23. Identification method according to Claim 22, characterised in that the medium or matrix contains at least one additional component that is necessary or advantageous for generating the signal.
24. Identification method according to Claim 23, characterised in that the at least one additional component is a catalyst, e.g. an enzyme or reaction partner for a reaction that leads to the formation of at least one reaction product that directly or indirectly generates a specific signal.
25. Identification method according to Claim 24, characterised in that the signal is generated by the combination of at least one reaction product with a suitable indicator system.
26. Identification method according to one of the Claims 1 - 25, characterised in that the signal is a visual signal.
27. Identification method according to Claim 26, characterised in that the signal is generated by means of inorganic and / or organic coloured compounds in a solid or dissolved state.
28. Identification method according to Claim 26 or 27, characterised in that released ions form coloured solutions, colloids or poorly soluble precipitates and in this way generate a specific signal.
29. Identification method according to Claim 26 or 27, characterised in that the signal is generated by the formation of charge-transfer complexes.
30. Identification method according to Claim 28, characterised in that the signal is generated by means of the sudden change in colour of an indicator, preferably a pH indicator.
31. Identification method according to one of the Claims 1 - 25, characterised in that the signal is perceived with the sense of smell or the sense of taste.

32. Identification method according to one of the Claims 1 - 30, characterised in that a signal is generated that can be evaluated by using suitable instruments.
33. Identification method according to one of the Claims 1 - 32, characterised in that a simple auxiliary device that is not an instrument is used for generating and / or evaluating the signal.
34. Identification method according to one of the Claims 1 - 33, characterised in that multiple different signals are generated at different locations on the object.
35. Identification method according to one of the Claims 1 - 34, characterised in that multiple different signals are generated simultaneously or staggered in time, and that the resulting complex signal pattern is evaluated.
36. Identification method according to one of the Claims 1 - 35, characterised in that the signal is or the signals are generated directly on the object.
37. Composition comprising one collective or multiple collectives of microcapsules and / or microparticles, which can be opened by the action of human saliva or constituents contained therein, in a liquid medium or matrix, wherein the composition contains all the components necessary for the generation of a signal, with the exception of the saliva constituents, in the microcapsules / microparticles and / or in the medium or matrix, wherein at least one of the components necessary for generating the signal is enclosed in the microcapsule or microparticle collective(s) in such a way that a signal can result only after the release of this component by the action of human saliva or constituents contained therein.
38. Composition according to Claim 37, characterised in that the microcapsules and / or microparticles comprise materials on the basis of poly-L-lysine, starch or chitosan or derivatives thereof.

39. Composition according to Claim 37 or 38, characterised in that all components that are necessary for generating the signal, with the exception of the saliva constituents, are enclosed in one or more microcapsule and / or microparticle collective(s).
40. Composition according to Claim 39, characterised in that it is a single component in a single microcapsule or microparticle collective.
41. Identification method for verifying the authenticity of an object, characterised in that the action of mechanical shearing forces and / or solvents on a marker that is bound to the object or contained therein generates a specific signal, and this signal is evaluated.
42. Identification method according to Claim 41, characterised in that the marker comprises microcapsules and / or microparticles which are opened by the action of mechanical shearing forces, so that at least one enclosed ingredient and / or bound constituent of the microcapsules and / or microparticles is released and directly or indirectly generates a specific signal.
43. Composition, comprising all components necessary for generating a signal as well as optionally a liquid medium or matrix, characterised in that a signal can result only after the action of mechanical shearing forces and / or solvents on the composition.
44. Composition according to Claim 43, comprising a collective or multiple collectives of microcapsules and / or microparticles, which can be opened by the action of mechanical shearing forces and / or solvents, in a liquid medium or matrix, wherein the composition contains, in the microcapsules / microparticles and / or in the medium or matrix, all components necessary for the generation of a signal, wherein, however, at least one component necessary for generating a signal is enclosed in the microcapsule or microparticle collective(s) in such a way that a signal can result only after the release of this component by the action of mechanical shearing forces and / or solvents.

45. Use of the composition according to Claims 43 and 44 for identification of the manipulation of an object marked with this composition.

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**New Patent Claims:**

41. Identification method for verifying the authenticity of an object, characterised in that the action of mechanical shearing forces on a marker that is bound to the object or contained therein generates a specific signal, and this signal is evaluated.
42. Identification method according to Claim 41, characterised in that the action of mechanical shearing forces and solvents on a marker that is bound to the object or contained therein generates a specific signal, and this signal is evaluated.
43. Identification method according to Claims 41 and 42, characterised in that the marker comprises microcapsules and / or microparticles which are opened by the action of mechanical shearing forces, so that at least one enclosed ingredient and / or bound constituent of the microcapsules and / or microparticles is released and directly or indirectly generates a specific signal.
44. Composition according to Claim 43, comprising a collective or multiple collectives of microcapsules and / or microparticles, which can be opened by the action of mechanical shearing forces, in a liquid medium or matrix, wherein the composition contains, in the microcapsules / microparticles and / or in the medium or matrix, all components necessary for the generation of a signal, wherein, however, at least one component necessary for generating the signal is enclosed in the microcapsule or microparticle collective(s) in such a way that a signal can result only after the release of this component by the action of mechanical shearing forces.
45. Composition according to Claim 44, characterised in that a signal can result only after the action of mechanical shearing forces and solvents on the composition.

46. Composition according to Claims 44 and 45, comprising a collective or multiple collectives of microcapsules and / or microparticles, which can be opened by the action of mechanical shearing forces, in a liquid medium or matrix, wherein the composition contains, in the microcapsules / microparticles and / or in the medium or matrix, all components necessary for the generation of a signal, whereby, however, at least one component necessary for generating a signal is enclosed in the microcapsule or microparticle collective(s) in such a way that a signal can result only after the release of this component by the action of mechanical shearing forces and / or solvents.
47. Use of the composition according to Claims 44 to 46 for identification of the manipulation of an object marked with this composition.